

CROSSING STUDIES IN *GUARDIOLA* (COMPOSITAE)

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Eleven of the twelve *Guardiola* species are closely related epappose species with opposite-leaved annual herbaceous stems produced from a perennial woody caudex. They are distributed in the western mountains of Mexico. Few qualitative characters are present in the heads, so the species are distinguished primarily on the basis of vegetative characters. The twelfth species (*G. pappifera*) is a pap-pose, lowland, herbaceous annual readily distinguished from the other species.

METHODS. Seven perennial species are represented in this study. Specimens of *G. arguta* did not flower so were not available for controlled laboratory experiments. The other four species (*G. pappifera*, *G. carinata*, *G. odontophylla*, and *G. odontophylla*) are known only by a total of six collections in various herbaria. I did not collect any of the four.

The plants used in this study were grown from field-collected seeds. The *Guardiola* achene has a very hard seed coat, and this combined with waterproofing properties of the seed coat and inner membranes retards the germination process. To obtain a rapid and high percentage of germination, achenes were placed on damp blotters in petri dishes and maintained in a warm (75-80°F), dark chamber for 2 or 3 days. After this time the seed coat and inner membranes were dissected from the embryo proper, and the excised embryo was returned to the incubation chamber. The embryos grew rapidly, and after 2-3 days the resulting seedlings were large enough to be planted in individual pots. Seedlings were maintained in the growth chamber until the stems were approximately 10 cm high, and then were transferred to the greenhouse. They were maintained in the greenhouse until buds formed, when they were returned to the laboratory for experimental procedures.

Guardiola ray florets, with only rare exceptions, are pistillate, the disk florets are staminate, and the plants were found to be completely self-incompatible. Therefore, emasculation was not necessary prior to hand pollination of the ray florets. Hand pollination was necessary because each head bears only 1-5 ray florets, so it was important to be sure that pollination had been accomplished, and to be as thrifty as possible in making crosses. At anthesis a disk floret was removed from a head with forceps; then under a dissecting microscope, the pollen was applied directly to stigmatic surfaces of the ray florets. This technique made possible the use of several disk florets of a head in different crosses. The heads were tagged and allowed to mature in the growth chamber or in the laboratory if the plants were too large for the growth chambers. Mature heads were

harvested and the achenes removed and germinated according to the procedures outlined above.

Production of a full-sized, apparently mature, fertile achene following hand pollination was considered a successful crossing attempt. Plants grown from seeds of these crosses were compared to parent plants.

Data for the crossing attempts are summarized in Table 1 and illustrated in Figure 1.

As an additional check on fertility of the parent plants, the offspring which did flower, and of some dried specimens, pollen stainability with cotton blue in lactophenol was investigated. Pollen stainability among the individual P₁ plants with two exceptions, viz. 78.6% and 83.7%, varied from 90.5% to 99.3%.

Of the progeny from intraspecific matings only five plants flowered. These had 71.1%, 87.9%, 92.9%, 95.9%, and 98.1% stainable pollen. Four progeny from the interspecific matings flowered and had 95.2%, 97.8%, 97.9%, and 98.0% stainable pollen.

OBSERVATIONS. Usually the ray florets emerged from the bud a day or two prior to the emergence of the disk florets. However, in many instances, apparently normal disk florets occupied the position of one or more ray florets in the outer ring of flowers and emerged with the ray florets.

The ray floret style with its two stigmatic branches is erect and white when receptive to pollen and will remain thus for about a week if not pollinated. If, however, the ray florets are pollinated with viable, compatible pollen, one can see a yellowing of the style begin to occur in about 6-10 hours (or in one case 3 1/2 hours) after pollination. After 24 hours the style and stigma are brown, withered, and withdrawn to the mouth of the tube of the ray corolla. If pollen used for the pollination is either incompatible or no longer viable, the ray stigma remains white and erect. Thus, about 24 hours after pollination I could read the previous day's crosses as to "take" or "no take". During the time I read this reaction to determine if it was more than coincidence, the reading of the reaction was correct in 196 of 232 instances (84.4%), establishing the generalization that the success of a cross can be determined after 24 hours. The majority of the incorrect determinations of crossing success were in cases where seeds were set subsequent to a "no take" reading.

In nature insects (We noticed primarily small Diptera on *Guardiola* heads.) pollinate the ray florets with pollen from the disk florets which bloom several days after the ray florets open. The insects may be attracted to the plants by the copious nectar produced by the very large nectaries of the disk florets.

Table 1. Summary of crosses attempted in *Guardiola*.

Seed Parent	Pollen Parent							Successes
	<i>G. angustifolia</i>	<i>G. mexicana</i>	<i>G. platyphylla</i>	<i>G. rosei</i>	<i>G. rotundifolia</i>	<i>G. thompsonii</i>	<i>G. tulocarpus</i>	
<i>G. angustifolia</i>	0:2(2)*	0:2	0	0:2	0:1	0:1	0:1	0:9
<i>G. mexicana</i>	0:1	32:56(10)	5:9	10:15	5:7	15:20	20:27	87:135
<i>G. platyphylla</i>	0	5:7	1:6(3)	5:7	2:3	7:8	4:5	24:36
<i>G. rosei</i>	1:1	3:4	5:8	8:20(5)	3:4	4:5	2:5	26:47
<i>G. rotundifolia</i>	0:1	1:2	1:1	2:2	2:6(3)	0	1:4	7:16
<i>G. thompsonii</i>	1:1	8:8	2:2	6:6	1:2	4:10(5)	6:8	28:37
<i>G. tulocarpus</i>	1:1	13:24	5:7	2:5	4:4	6:10	10:30(11)	41:81
Successes	3:7	62:103	19:33	33:57	17:27	36:54	43:80	213:361

*0:2(2) is read as no successes in 2 attempts with 2 of the attempts being selfs.

Total successes, all attempts

213:361 59.0%

Successes excluding 39 selfs

213:322 66.1%

Successes of interspecific attempts

156:231 67.5%

Successes of intraspecific attempts excluding selfs

57:91 62.6%

Table 2. Summary of time required for maturation of seeds in *Guardiola*.

	Broad-leaved Seed Parent			Narrow-leaved Seed Parent			Total
	Broad-leaved Pollen Parent	Narrow-leaved Pollen Parent	Total	Broad-leaved Pollen Parent	Narrow-leaved Pollen Parent	Total	
9			0		1	1	1
10			0		1	1	1
11			0		1	1	1
12			0		0	0	0
13			0		0	0	0
14			0		1	1	1
15			0		2	2	2
16			0		6	6	6
17			0	10	5	15	15
18			0	8	16	24	24
19			0	2	15	17	17
20	1		1	6	6	12	12
21	6		6	6	7	13	13
22	4	1	5	5	8	13	13
23	2	3	5	0	1	1	1
24	4	0	4	5	0	5	5
25	6	3	9	3	3	6	6
26	1	2	3	2	0	2	2
27	0	0	0	0	2	2	2
28	1	0	1	0	0	0	0
Mean Days to Mature	23.20	24.22	23.5	20.32	18.97	19.5	19.5

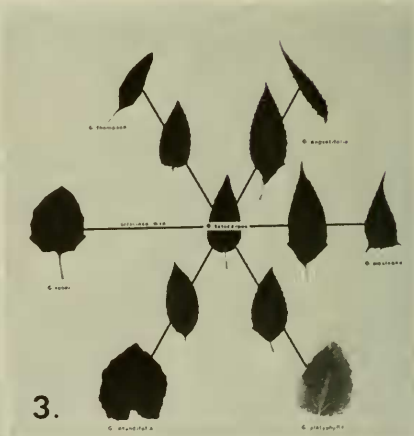
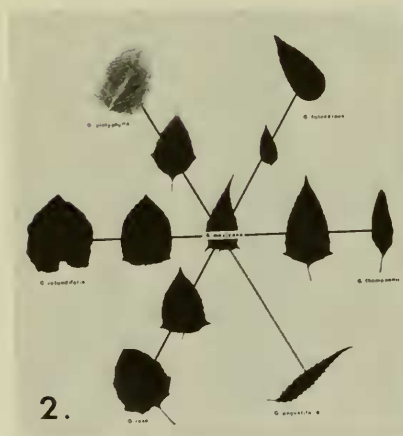
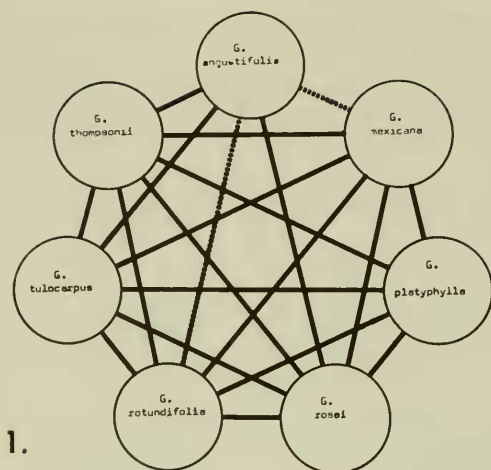


Figure 1. Experimental crosses in *Guardiola*. Heavy lines indicate successful seed set. Broken lines indicate crosses attempted but no seeds set. Figure 2. Experimental crosses using *G. mexicana* as the seed parent. A typical leaf of the seed parent is in the center of the figure. Typical leaves of pollen parents are around the periphery. Typical leaves of the F_1 obtained between the parents. Figure 3. Experimental crosses using *G. tulocarpus* as the seed parent.

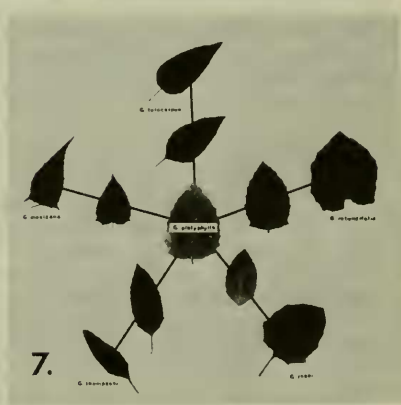
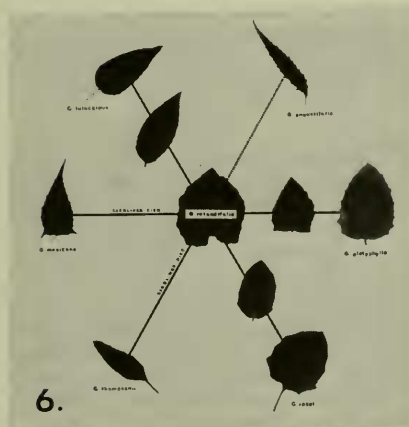
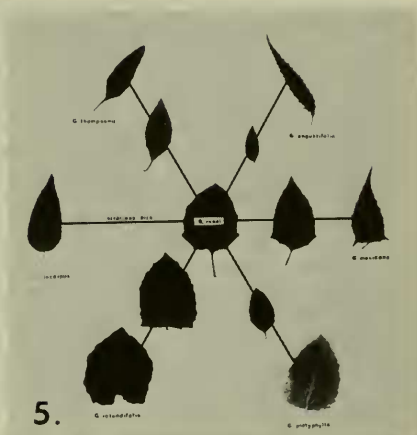
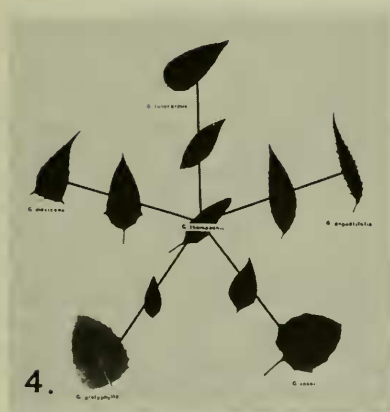


Figure 4. Experimental crosses using *G. thompsonii* as the seed parent. Figure 5. Experimental crosses using *G. rosei* as the seed parent. Figure 6. Experimental crosses using *G. rotundifolia* as the seed parent. Figure 7. Experimental crosses using *G. platyphylla* as the seed parent.

The disk florets are touch-sensitive when they mature. When touched, the style emerges a little from the tube formed by the connate anthers and carries some pollen out with it. At the same time the floret describes a small circle. Stamen irritability is also reported in *Arnica* by Vuilleumier (1969) and Small (1915) who state that there are two basic types of stamen irritability. One involves elongation of the style and the second, contraction of the filaments, causes tilting of the floret toward the tactile stimulus. Hoffman (1894) also noted filament irritability in some Cynareae, especially *Centaurea*, and in *Perezia multiflora* Less. and *Trixis discolor* Gill of the Mutisieae. The mechanism involved in *Guardiola*, whether elongation of the style or contraction of the filaments, is not known at this time. However, on occasion it was noted that the style of the ray florets also exhibited this movement. Therefore, it seems likely that stylar elongation, perhaps due to release of tension, is responsible for the pushing out of the pollen, and that the circular movement of the entire disk floret is due to sequential contraction of the filaments. Thus, when an insect lands on a mature disk floret, presumably to obtain nectar, it touches the style, thus initiating the circular movement of the floret, and a little pollen is pushed out and may be rubbed onto the ventral surface of the insect. When the insect then investigates a ray floret it may transfer the pollen to the ray stigma, thus effecting pollination. The disk floret requires about 15-30 minutes to recover from the touch-stimulated movement, and may repeat the process.

Table 1 shows that of 361 total crosses attempted, 213 or 59% were successful; that is, set seed. There were 39 attempts, none of which were successful, to self plants. If these selfing attempts are removed from the total attempts, then 213 of 322 attempts or 66.1% were successful. The success rates of interspecific (67.5%) and intraspecific (62.6%) were essentially the same. Successful seed set apparently depended on the condition of the plant at the time of pollination and on the viability of the pollen used. *Guardiola* pollen appears to be viable, and sticky, for only two or three days. Some crosses were made deliberately using pollen which was powdery and no longer sticky as it is when fresh. No seeds were set under these circumstances, and it is assumed that the pollen retains its viability for a rather short period of time. Some plants would successfully set seed following pollination with pollen from almost any source and then suddenly stop setting seeds for no apparent reason. Light level may have been important in some circumstances, for I had poor success with plants which were too tall to fit into the growth chamber and were maintained under reduced light conditions. Plants of *Guardiola angustifolia* were especially problematical because when a budding plant was brought into the laboratory from the greenhouse, the buds would abort before flowering.

The time required for maturation of the seeds (from pollination to release of mature seeds) varies considerably; broad-leaved and narrow-leaved species differ (Table 2). When a broad-leaved plant was the seed parent, seed maturation averaged 23.5 days with a median

of 23.5 days, and a range of 20-28 days. When a narrow-leaved plant was the seed parent, seed maturation averaged 19.5 days with a median of 19 days and a range from the remarkably short time of 9 days to 27 days.

Mean maturation time for crosses involving two broad-leaved parents was 23.20 days, while that for two narrow-leaved parents was 18.97 days. A t-test comparison of these means indicates that the difference is highly significant at the 5% confidence level. This data supports the contention of evolutionary divergence of those two species groups.

A t-test comparison of the influence of the pollen parent on the mean days required for seed maturation indicated acceptance of the means as the same at the 5% confidence level when a broad-leaved seed parent is involved and at the 2% confidence level when a narrow-leaved seed parent is involved in the cross. This suggests that the source of the pollen used does not significantly alter the mean days required for seed maturation.

Similar comparisons of the influence of the seed parent on the time required for seed maturation indicate highly significant differences at the 5% confidence level for both broad-leaved and narrow-leaved species groups. This suggests a strong seed parent influence on the time required for seed maturation.

Success in establishing hybrid plants in nature depends on seed viability, seedling vitality, and ability of the F₁ plants to reproduce. Viability of seeds set in these experiments was 96.1%. The embryo in apparently fertile seeds which did not germinate was either malformed or absent.

Approximately 75% of the F₁ seedlings survived at least long enough to produce plants of flowering size. Non-survival of the seedlings was due largely to unknown causes, but also contributing were greenhouse pests which destroyed a number of seedlings, and malfunctioning growth chambers, which on two occasions refrigerated to about 2°C, a temperature below the tolerance limits of some of the seedlings.

Very few of the surviving F₁ plants, many of which were nine months old, flowered under greenhouse conditions. Under similar conditions, plants grown from field-collected seeds flowered in 3 1/2 to 5 months.

INTRASPECIFIC MATING ATTEMPTS. Seed set occurred in 62.6% of the intraspecific mating attempts (Table 1). There were successful intraspecific matings within all *Guardiola* species studied except *G. angustifolia* in which the few florets available were all used in interspecific mating attempts.

Seeds produced from the intraspecific matings germinated. The resulting seedlings grew readily to flowering size. As expected, the range of variation of these plants was within the range of variation of these plants was within the range of variation of the parent species. However, only a few buds were produced by these progeny. Some of the buds were sacrificed in order to examine meiosis, in which pairing appeared normal. The rest of the buds were allowed to flower for use in various mating attempts, none of which were successful.

INTERSPECIFIC MATING ATTEMPTS. Interspecific mating attempts were 67.5% successful (Table 1), essentially the same as in the intraspecific matings.

There were no successful matings involving *G. angustifolia* as the seed parent. However, the 60% success rate when *G. angustifolia* was used as the pollen parent suggests that the problems were physical rather than genetic when *G. angustifolia* was used as the seed parent. Otherwise there was no clear pattern of success or failure.

Seeds resulting from the various interspecific matings were germinated and grew readily to flowering size. The plants varied, but as expected, were intermediate between the parental species. Figures 2-7 illustrate typical leaves from parent plants used in the crossing experiments and pictorially describe the leaves of the hybrid plants derived from the crosses. Almost none of these plants set flowers, but in those which did, meiotic pairing appeared normal. A few buds were allowed to flower and all mating attempts using them as either pollen or seed parent failed. While the sample is small, it suggests a fertility barrier in the progeny, a barrier which would prevent reproduction by any hybrid plant which might occur in nature and thus support the integrity of the various species. Because of the small sample, further work is necessary in order to make certain that such a reproductive barrier exists and what may be its nature.

LITERATURE CITED

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